



Certificate of Analysis

Standard Reference Material[®] 2365

BK Virus DNA Quantitative Standard

This Standard Reference Material (SRM) is intended for use in the value assignment of BK virus deoxyribonucleic acid (DNA) quantitation materials, primarily those used for quantitative polymerase chain reaction (qPCR). SRM 2365 consists of a well-characterized, linearized plasmid, containing BK virus DNA solubilized in 10 mmol/L 2-amino-2-(hydroxymethyl)-1,3 propanediol hydrochloride (Tris HCl) and 1 mmol/L ethylenediaminetetraacetic acid disodium salt (disodium EDTA) pH 8.0 buffer (TE), with 50 ng/ μ L yeast tRNA added to ensure stability. A unit of the SRM consists of one 0.5 mL tube containing approximately 110 μ L of DNA solution. The tube is labeled and is sealed with a screw cap.

Certified Values: Certified values are provided in Table 1. A NIST certified value is a value for which NIST has the highest confidence in that all known or suspected sources of bias have been accounted for. The copy number values are metrologically traceable to the natural units count 1 and ratio 1 and International System of Units (SI) derived units of volume.

Table 1. Certified Value for SRM 2365

Analyte	Certified Value (copies/ μ L)	95% Probability Uncertainty Interval (copies/ μ L)	Standard Uncertainty, $u(X)$ (copies/ μ L)	Effective Coefficient of Variation, $CV=100 \times u(X)/X$
BK Virus DNA copy number	558,000	534,000 to 582,000	12,000	2.2%

Expiration of Certification: The certification of **SRM 2365** is valid, within the stated measurement uncertainties, until **15 July 2023**, provided the SRM is handled and stored in accordance with the instructions given in this certificate (see “Instructions for Storage and Use”). The certification is nullified if the SRM is damaged, contaminated, or otherwise modified.

Maintenance of SRM Certification: NIST will monitor this SRM over the period of its certification. If substantive technical changes occur that affect the certification before the expiration of this certificate, NIST will notify the purchaser. Registration (see attached sheet or register online) will facilitate notification.

Coordination of the technical measurements and analysis leading to the certification was under the direction of M. Cleveland of the NIST Biomolecular Measurement Division.

Statistical consultation for this SRM was provided by B. Toman of the NIST Statistical Engineering Division.

Support aspects involved in the issuance of this SRM were coordinated through the NIST Office of Reference Materials.

Michael J. Tarlov, Chief
Biomolecular Measurement Division

Gaithersburg, MD 20899
Certificate Issue Date: 11 September 2018

Steven J. Choquette, Director
Office of Reference Materials

Storage and Handling: Until required for use, SRM 2365 should be stored in the dark between 2 °C and 8 °C.

The screw-cap vial should be mixed briefly and centrifuged (without opening the vial cap) prior to removing sample aliquots for analysis. For the certified values to be applicable, materials should be withdrawn immediately after opening the vials and processed without delay. Certified values do not apply to any material remaining in recapped vials. The certification only applies to the initial use and the same results are not guaranteed if the remaining material is used at a later date.

Use: For qPCR assays, value assign secondary standard DNA solutions relative to the certified copy number concentrations. Calibrate the assays to SRM 2365 using one or more dilution series prepared from SRM 2365. Users should keep in mind that a potential for pipetting error exists; therefore, suitable care should be exercised in preparing calibration solutions.

Safety: SRM 2365 is a bacterial DNA source material. Handle SRM 2365 as a Biosafety Level 1 material capable of transmitting infectious disease. SRM 2365 should be disposed to in accordance with local, state, and federal regulations.

Source: The BK Virus genome construct (NCBI accession # JQ713822.1) was synthesized and cloned into a pUC57 plasmid. Restriction sites (AhdI and BssHII) were added on either side of the BK Virus DNA, to allow the BK genome region to be isolated from the rest of the plasmid. The plasmid was transformed into *E. coli* Sure2 cells. The plasmid was grown in a 2.5 L culture, purified, and linearized with restriction enzymes (AhdI and BssHII). The linearized material was then filtered to remove precipitate from bovine serum antigen (a component in the restriction digests). BK Virus DNA solution was combined with TE buffer, and 50 ng/μL yeast tRNA was added to ensure stability.

Additional Information: Full details on the production, analysis, and statistical evaluation of SRM 2365 are provided in NIST Special Publication 260-191, *Certification of Standard Reference Material® 2365 BK Virus DNA Quantitative Standard* [1].

REFERENCES

- [1] Cleveland, M.H.; Farkas, N.; Kiesler, K.M.; Toman, B.; Vallone, P.M.; *Certification of Standard Reference Material® 2365 BK Virus DNA Quantitative Standard*. NIST Special Publication 260-191, available at: <https://doi.org/10.6028/NIST.SP.260-191> (accessed Sep 2018).

Users of this SRM should ensure that the Certificate of Analysis in their possession is current. This can be accomplished by contacting the SRM Program: telephone (301) 975-2200; fax (301) 948-3730; e-mail srminfo@nist.gov; or via the Internet at <https://www.nist.gov/srm>.